INTERNATIONAL JOURNAL OF PLANT PROTECTION
VOLUME 7 | ISSUE 2 | OCTOBER, 2014 | 312-317 ● e ISSN-0976-6855 | Visit us : *www.researchjournal.co.in*

IJPP

RESEARCH PAPER DOI : 10.15740/HAS/IJPP/7.2/312-317

Studies on the interaction between *Odoiporous longicollis* and endophytic *Beauveria bassiana* by establishing fungal infection to bsw in the plant system

■ M.K. PRABHAVATHI* **AND** S.K. GHOSH

Department of Entomology, University of Agricultural Sciences, GKVK, BANGALORE (KARNATAKA) INDIA

ARITCLE **I**NFO

Received : 24.03.2014
Revised : 23.07.2014
Accepted : 07.08.2014

KEY **W**ORDS :
Beauveria bassiana Balsamo, *Odoiporous longicollis*

***Corresponding author:**
Email: prabhavathient@gmail.com

ABSTRACT

Beauveria bassiana, which is effective entomopathogen against a variety of insect pests, is the most researched and commercialised fungal biopesticide. Laboratory and screenhouse studies have revealed great potential of this entomopathogenic fungus for use against the banana weevil, *Odoiporous longicollis*, in banana. Result revealed that *B. bassiana* can colonise internal banana tissues for at least four months after tissue-cultured plantlets are dipped in a spore suspension. The type of banana cultivar did not affect colonisation by *Beauveria bassiana* and, even when elevated *B. bassiana* doses were used, plant growth was not reduced. In a set of three screen house experiments, weevil mycosis rates in *B. bassiana*-treated plants were 50-70 per cent within 10 – 15 days when the adult weevils were allowed to infest the banana plantlets having *B.bassiana* as a endophyte in their systems prior to infest. And the presence of the fungus inside treated plants led to a reduction in pseudostem weevil damage up to >50 per cent. Application of *B. bassiana* as an artificial endophyte inside banana plants could circumvent bottlenecks associated with its application as a conventional biopesticide, because (i) it kills the damaging larval stages inside the plant, (ii) it is protected from adverse biotic and abiotic factors, (iii) little inoculum is required, drastically reducing its cost, and (iv) farmers do not need to apply the biological control organism themselves, as the technology is easily transferable to a commercial tissue culture producer.

How to view point the article : Prabhavathi, M.K. and Ghosh, S.K. (2014). Studies on the interaction between *Odoiporous longicollis* and endophytic *Beauveria bassiana* by establishing fungal infection to BSW in the plant system. *Internat. J. Plant Protec.*, **7**(2) : 312-317.

INTRODUCTION

Bananas and plantains (*Musa* spp.) are the key components in food security and agricultural sustainability, and a source of income to the resource poor farmers around the East African highland region. However, the production of bananas and plantains in East Africa has been steadily declining due to several constraints, including pests and diseases. The banana weevil, *Odoiporous longicollis* (Coleoptera: Curculionidae) remains the primary arthropod pest of bananas and plantains. It is estimated that banana pseudostem borer causes 10-90 per cent yield loss depending on the growth stage of the crop and management efficiency (Thippaiah *et al*., 2011). Unfortunately, because the adult weevils are mostly concealed in soil, and the larvae are protected within the banana rhizome and pseudostem, control by conventional insecticides, cultural practices or classical biological control methods has proven challenging and impractical (Treverrow *et al.*, 1993).Whereas factors such as resistance, high cost and environmental pollution have hampered the use of insecticides (Collins *et al*., 1991 and Gold *et al*., 1999), cultural practices, including trapping, good crop husbandry and clean planting materials are labour-intensive (Gold *et al*., 2001 and Masanza *et al*., 2005). Furthermore, attempts for biological control using exotic natural enemies have had only limited impact (Koppenhöfer and Schmutterer, 1993), while few banana or plantain cultivars provide tolerance or resistance against *Odoiporous longicollis*.

The endophytic entomopathogenic fungus, *Beauveria bassiana* (Balsamao) Vuillemin has been researched extensively as another option for controlling many insect pests including the banana weevil (Kaaya *et al.,* 1993; Nankinga, 1999; Godonou *et al.,* 2000 and Schoeman and Botha, 2003). Fungi that occur inside asymptomatic plant tissues are known as fungal endophytes.

MATERIAL AND METHODS

Collection of banana pseudostem weevil :

In all the gardens the split-pseudostem pieces measuring 45-50 cm in length were kept and covered with trash besides the banana plants and pseudostem traps were installed. Pseudostem traps consists in pieces of pseudostem with 50 cm long cut in the middle lengthwise. The traps, placed at the base of the plants, were inspected every 10 days and the adults collected were transferred to the laboratory. Later, counted and mass reared in the laboratory. The weevils were mass multiplied to study the mortality per cent in *B. bassiana* inoculated banana plants.

Interaction of *Odoiporous longicollis* and endophytic, *B. bassiana* in the banana plants :

Later allowed the banana pseudostem weevils (3 weevils/plant) to fungal established plants (60 days after inoculation of fungus) for different methods (dipping, injection and rice substrate methods). After releasing the weevils, pots were covered by cages to avoid escape of allowed weevils, then assessed the insect mortality on a daily basis and allowed the same number of weevils into controlled plants and recorded the observation on per cent damage to the plants by banana pseudostem weevil and recorded the effect of *B. bassiana* on weevil (% mortality of weevil), dead weevils were collected and sterilized by dipping in 5 per cent sodium hypochlorite and 3-4 times by distilled water and plated on the humid chamber (absorbent cotton and tissue paper were kept in sterilized petri plate and socked by the water and then glass slide was kept on tissue paper, after these, dead weevils were kept in Petri plate contained glass slide). Then kept it in incubator, after one week, the fungal outgrowth was observed on banana (white dense mycelium) weevil. Then recorded the plant parameter observation at weekly intervals.

Per cent weevil mortality was recorded by using formula:

$$\frac{\textbf{Number of mycosis weevil}}{\textbf{Total number of exposed weevil}} \times \textbf{100}$$

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads :

Rearing of *O. longicollis* :

O. longicollis was collected from different banana infested fields (plate1) and reared in laboratory at 26±1°C.

Egg:

The incubation period ranged from 3 to 8 days.

Larvae:

The grub completed five instars.

The first instar lasted for 2 to 4 days.

The second instar took 3 to 5 days.

The third instar lasted for 4.8 to 6 days.

The fourth instar lasted for 6.5 to 7.5 days.

The fifth instar was lasted for 12 to 14 days.

The total larval period varied from 30 to 35 days during summer and 50 to 60 days during winter.

Cocoon :

Larva constructed a cocoon using fibres, which are arranged spirally. It measured 27 to 36 mm in length and breadth ranged from 9 to 11 mm.

Pupa :

The pupa was exarate type, The pupal period lasted for 17 to 22 days.

Adult :

The adult weevil was reddish brown in colour and turned black before emergence. The body length of male and female was 13 to 16.5 mm and 15.5 to 17.5 mm, respectively. The breadth was 2.9 to 3.6 mm and 3.2 to 4.2 mm, respectively. Adult longevity was 50 to 60 days during summer and 75 to 95 days during winter. The sexes could be separated on the basis of rostral characters. Rostral punctuation in case of male was larger in size and raised, giving the rostral surface a more or less rough appearance. In case of female, the rostral punctuation was small sized and there was no raised area on rostral surface.

The total life cycle of *O. longicollis* from egg to adult emergence varied from 53 to 65 days during summer and 75 to 95 days during winter.

Mortality of weevil due to endophytic *B. bassiana* :

After 45 days of *B. bassiana* inoculation, banana pseudostem weevils were released into pots. Mortality of weevil was observed in tissue culture banana plants due to endophytic action of *B. bassiana.*

The mortality of weevil significantly differed in different methods of *B. bassiana* inoculation. The highest weevil mortality was recorded in dipping method, followed by injection method and least was recorded in solid substrate and no mortality of weevils was recorded in controlled banana plants (73.33%, 46.66%, 33.33% and 0%, respectively) (Fig. 1).

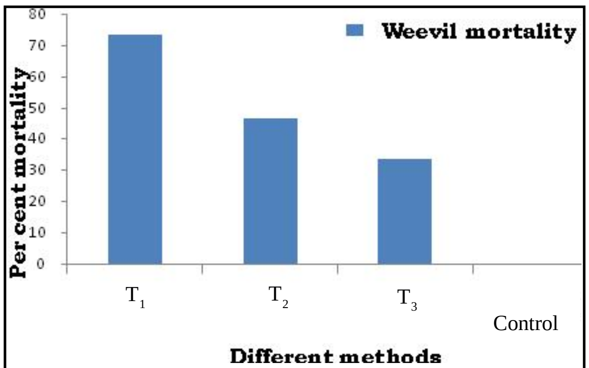

Fig. 1 : Mortality of weevil

The LC_{50} value of *B. bassiana* calculated was 1.06×10^{12} spore/ml with fiducial limit 5.72×10^{10} to 1.196×10^{36} spore/ml. LT_{50} value for weevil reared with *B. bassiana* in root dipping, injection and solid substrate were 25.24, 15. 63 and 10.22, respectively (Fig. 2, 3 and 4).

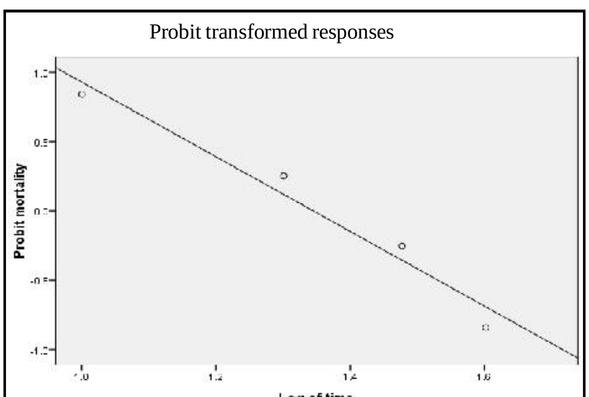

Fig. 2 : Regression time on probit mortality of weevil in dipping method

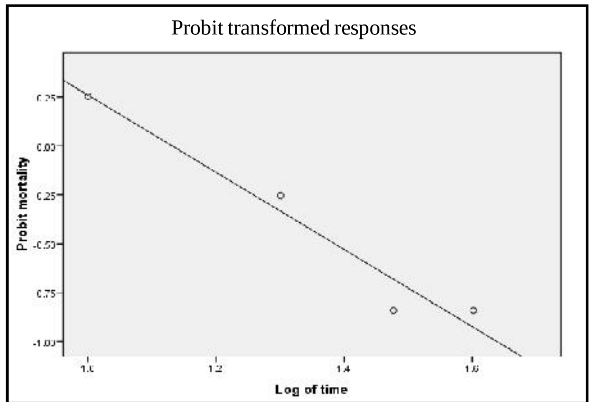

Fig. 3 : Regression time on probit mortality of weevil in injection method

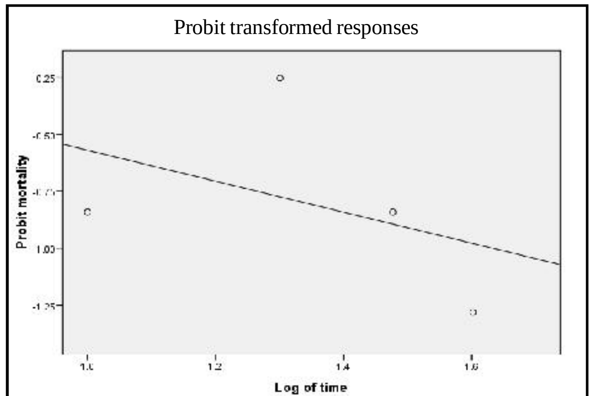

Fig. 4 : Regression time on probit mortality of weevil in solid substrate method

It was evident that from the plot of regression, as the concentrations of spore suspension increases there was

Table 1 : Mortality of weevil in dipping method

Number of days/times	Number of weevil released	Number of weevils infected with *B. bassiana*	Per cent mortality (Mean)
5-10	15	5.1	33.33 (35.24)a
11-15	15	3.00	20 (26.55)b
16-20	15	1.00	6.66 (14.95)c
21-25	15	0	0 (0.00)d
26-30	15	1.00	6.66 (14.95)c
31-35	15	1.00	6.66 (14.95)c
36-41	15	0	0 (0.00)d
41-45	15	0	0 (0.00)d
			**
S.E. ±			0.41
C.D. (P=0.05)			1.24

*Significant, Figures in the parentheses are arc sign transformed values by DMRT treatment means in the same columns by common superscript are not significantly different

Table 2 : Mortality of weevil in injection method

Number of days	Number of weevil released	Number of weevils infected with *B. bassiana*	Per cent mortality (Mean)
5-10	15	2.00	13.33 (21.40)a
11-15	15	2.1	14 (21.40)a
16-20	15	1.1	7.33 (15.70)b
21-25	15	1.00	6.66 (14.95)b
26-30	15	0.5	3.33 (10.51)c
31-35	15	1	6.66 (14.95)b
36-41	15	0	0 (0.18)c
41-45	15	0	0 (0.18)c
F-test			*
S.E. ±			(0.22)
C.D. (P=0.05)			(0.67)

*Significant, Figures in the parentheses are arc sign transformed values by DMRT treatment means in the same columns by common superscript are not significantly different.

Table 3 : Mortality of weevil in solid substrate method

Number of days	Number of weevils released	Number of weevils infected with *B. bassiana*	% Mortality (Mean)
05-10	15	0.6	4 (0.18)c
11-15	15	2.00	13.33 (21.40)a
16-20	15	1.1	7.33 (15.70)b
21-25	15	0.5	3.33 (10.51)c
26-30	15	-	0 (0.18)c
31-35	15	1.00	6.66 (14.95)b
36-41	15	1.00	6.66 (14.95)b
41-45	15	-	0 (0.18)
F-test			*
S.E. ±			(0.18)
C.D. = (P=0.05)			(0.54)

*Significant, Figures in the parentheses are arc sign transformed values by DMRT treatment means in the same columns by common superscript are not significantly different

increases in the mortality. The Chi-squre test showed homogeneity of the test population which is reflection of a goodness of fit.

Mortality of weevil due to endophytic *B. bassiana* infestation in dipping method (1.5×10^9/ml) :

In dipping method, the mortality of weevil significantly differed on different days. The highest mortality (33.33%) was recorded at 5 to10 days after releasing of banana pseudostem weevils and it was followed by 11 to 15 days and the least mortality was observed in 16 to 20 days and it was found on par with 26 to 30 and 31 to 35 and no mortality was recorded in 21 to 25 and at 36 days onwards (Table 1).

Mortality of weevil due to *B. bassiana* infestation in injection method (1×10$^{9/ml}$) :

In injection method the mortality of weevil significantly differed at different days. The highest mortality was recorded at 11 to 15 days after releasing of banana pseudostem weevil and the least mortality was recorded in 26 to 30 days and no mortality was recorded in 41 to 45 days (Table 2).

Mortality of weevil due to *B. bassiana* infestation in solid substrate method (1.5% of 1 × 10^9/g) :

In solid substrate method, the mortality of weevil significantly differed on different days. The highest mortality was recorded at 11 to 15 days after releasing of banana pseudostem weevil and the least mortality was recorded in 21 to 25 days. Least mortality was recorded in 21 to 25 days and no mortality in 26 to 30 days, 41 days onwards was recorded.

B. bassiana is an entomopathogenic fungus which is highly virulent in nature to development and use because being an environment friendly bioinsecticide. Many reports describes the importance of *B. bassiana* as biological insecticide for the control of number of pests such as termites, thrips, whiteflies, aphids and beetles like banana pseudostem weevil, rhizome weevil, root grub, red palm weevil etc. Fungal entomopathogens have been isolated as endophytes from several plants which manage insect pests and minimize the damaging level to the plants. Current research efforts have directed to use of entomopathogenic fungi as endophyte in the plant system and it is protected from adverse biotic and abiotic factors, little inoculum is required for the management of PSW thereby it drastically reduces its cost. In this study, an attempt has been made on mass rearing of banana pseudostem weevil, mass production of entomopathogen, *B. bassiana*, and establishment of *B. bassiana* as artificial endophyte in banana ecosystem, pathogenicity and effective dosage on weevil under glass house condition.

The mortality of weevil was observed in *B. bassiana* treated banana plants due to the toxins of the fungus, Beauvericin, against weevil and due to the presence of fungal metabolites that cause feeding deterrence or antibiosis (De Hoog, 1972).

Vega *et al.* (2008) reported *B. bassiana* as a maize endophyte suggesting that the reduced tunneling of *O. nubilalis* could be due to the presence of fungal metabolites that cause feeding deterrence or antibiosis. This is based on the overwhelming absence of *B. bassiana* infection within *O. nubilalis* individuals that feed on endophytic plants (Lewis and Bing, 1991 and Bing and Lewis, 1992 and 1993), that showed 2.5 per cent mycosis on insects feeding on endophytic plants (and 1.7% mycoses on insects feeding on plants with no *B. bassiana*). When spores *B. bassiana* come in contact with the cuticle (skin) of susceptible insects, it germinates and grow directly through the cuticle to the inner body of the host. Here, the fungus proliferates throughout the insect's body, producing toxins and draining the insect nutrients, eventually killing it.

Akello *et al*. (2008) reported after 15 weeks, the presence of *B. bassiana* as an endophyte in banana tissues and greatly reduced banana weevil populations and their damage to plants. Between 53.4 and 57.7 per cent of the banana weevil adults died because of *B. bassiana* infection, resulting in a reduction of plant damage by 29.1 to 62.7 per cent depending on plant part. The weevil mortality significantly differed in different methods of inoculation that is, the highest weevil mortality was observed in dipping method of *B. bassiana* (73%) as compared to injection (51%) and solid substrate (41%) method and weevil mortality was not observed in case of control. This was due to the colonization of fungus being highest in case of dipping method as compared to other method and no colonization was observed in case of control. This showed that the colonization of *B. bassiana* was directly proportion to the weevil mortality and the weevil mortality was decreased over the times.

Akello *et al*. (2007) reported that in laboratory bioassays, the fungus was highly pathogenic to *C. sordidus*, causing more than 90 per cent mortality within two weeks, 48 per cent and 20 per cent of weevil mortality one and five month after *Beauveria* inoculation. Magara *et al*. (2003) obtained the highest mortality (30-70%) for weevils released in pots immediately after *B. bassiana* application. However, mortality steadily decreased (5-40%) for weevils released 90 days after applying *B. bassiana*. It was due to the efficacy of *B. bassiana* which decreases over the time.

The highest mortality of pseudostem weevil in banana plants due to endophytic *B. bassiana* in was recorded in root and rhizome dipping method, followed by injection method and lowest was in solid substate method and no mortality was recorded in control plants.

REFERENCES

Akello, J., Dubois, T., Coyne, D., Gold, C. S. and Kyamanywa, S. (2007). Colonization and persistence of the entomopathogenic fungus, *Beauveria bassiana*, in tissue culture of banana. *African Crop Sci. Confr. Proceedings*, **8**: 857-861.

Akello, J., Dubois, T., Coyne, D., Kyamanywa, S. (2008). Effect of endophytic *Beauveria bassiana* on populations of the banana weevil, *Cosmopolites sordidus*,and their damage in tissue-cultured banana plants. *Entomol. Exp. Appl.*, **129**: 157–165.

Bing, L.A. and Lewis,L.C. (1991). Suppression of *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) by endophytic [*Beauveria bassiana* (Bals.) Vuill]. *Environ. Entomol.*, **20**: 1207–1211.

Bing, L.A. and Lewis, L.C. (1992). Endophytic *Beauveria bassiana* (Balsamo) Vuillemin in corn: the influence of plant growth stage and *Ostrinia nubilalis* (HiJbner). *Biocontrol Sci. Technol.*, **2**: 39-47.

Bing, L.A. and Lewis, L.C. (1993). Occurrence of the entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin in different tillage regimes and in *Zea mays* L. and virulence towards Ostrinia nubilalis (Hübner). *Agric. Ecosyst. Environ*., **45** : 147–156.

Collins, P.J., Treverrow, N.L. and Lambkin, T.M. (1991). Organophosphous insecticide resistance and its management in the banana weevil borer, *Cosmopolites sordidus* (Ger.) (Coleoptera: Curculionidae) in Australia. *Crop Protection*, **10**: 215-221.

De Hoog, G.S. (1972). The genera *Beauveria, Isaria, Tritirachium* and *Acrodontium gen*. nov. *Studies Mycol*., **1** : 1–41.

Godonou, I., Green, K.R., Oduro, K.A., Lomer, C.J. and Afreh, N.K. (2000). Field evaluation of selected formulations of *Beauveria bassiana* for the management of the banana weevil (*Cosmopolites sordidus*) on plantain. *Biocontrol Sci. Technol.*, **10**: 779-788.

Gold, C.S., Karamura, E.B., Kiggundu, A. and Abera, A.M.K. (1999). Geographic shifts in highland cooking banana (*Musa* spp., group AAA-EA) production in Uganda. *Inter. J. Sustainable Develop. World Ecol.*, **6**: 45-59.

Gold, C.S., Pena, J.E. and Karamura, E.B. (2001). Biology and integrated pest management for the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), *Inter. Pest Manage. Rev.*, **6**: 79-155.

Kaaya, G.P., Seshu-Reddy, K.V., Kokwaro, E.D. and Munyinyi, D.M. (1993). Pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae* and *Serrratia marcescers* to the banana weevil, *Cosmopolites sordidus. Biocontrol News & Information*, **3**: 177-187.

Koppenhoffer, A.M. and Schmutterer, H. (1993). Dactylosternum abdominal (F.) (Coleoptera: Hydrophilidae): A predator of the banana weevil. *Biocontrol Sci. Technol.*, **3**: 141-147.

Lewis, L.C. and Bing, I.A. (1991). *Bacillus thuringiensis* Berliner and *Beauveria bassiana* (Bals.) Vuillemen for European corn borer control: Potential for immediate and season-long suppression. *Can. Entomol.*, **123**: 387–393.

Magara, E., Nankinga, C.M., Gold, C.S., Kyamanywa, S., Tushemereirwe, W.K., Moore, D., Gowen S.R. and Ragama, P. (2003). Influence of soil amendments in the delivery of *Beauveria bassiana* for control of the banana weevil, *Cosmopolites sordidus* (Ger.). *African Crop Science Conference Proceedings*, **6**: 205-209.

Masanza, M., Gold, C.S. and Van, Huis A. (2005). Distribution, timing of attack, and oviposition of the banana weevil, *Cosmopolites sordidus*, on banana crop residues in uganda. *Entomologia Experimentaliset Applicata*, **117**: 119-126.

Nankinga, C.M. (1999). Characterization of entomopathogenic fungi and evaluation of delivery systems of *Beauvaria bassiana* for the biological control of the banana weevil, *Cosmopolites sordidus*. Ph.D Thesis, University Reading. UK, 276 pp.

Schoeman, P. S. and Botha, H. (2003). Field management of the banana weevil *Cosmopolites sordidus* (Coleoptera: Curculionidae) with *Beauveria bassiana. African Pl. Prot.*, **9**: 1-3.

Thippaiah, M., Kumar, Ashok, C.T., Shivaraju, C., Kumar, Sudhir, S. and Naveena, N.L. (2011). Study of biology of banana pseudostem weevil, *Odoiporus longicollis* Oliv. *Internat. J. Entomol.*, **2**(1): 1-5.

Vega, F.E., Posada, F., Aime, M.C., Pava-Ripoll, M., Infante, F. and Rehner, S.A. (2008). Entomopathogenic fungal endophytes. *Biol. Control,* **46**: 72–82.

Treverrow, N.L. and Bedding, R.A. (1993). Development of a system for the control of the banana weevil borer, *Cosmopolites sordidus* with entomopathogenic nematodes. In: Bedding, R., Akhurst, R., Kaya, H. (Ed.). *Nematodes and the biological control of insect pests*. East Melbourne: *CSIRO,* **6** : 41-47.

7th Year
★★★★★ of Excellence ★★★★★